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Atty. Docket No. 085761-0004

"Express Mail" Label No. EL140093388US

Date of Deposit September 18, 1998

BOX PATENT APPLICATION
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By _____

Sir:

Transmitted herewith for filing under 37 CFR §1.53(b) is the
[x] patent application of TAKATSUJI, et al.
[ ] continuation patent application of
[ ] divisional patent application of
[ ] continuation-in-part patent application of

Inventor(s)/Applicant Identifier: Hiroshi TAKATSUJI and Hitoshi NAKAGAWA

For: METHOD FOR SHORTENING INTERNODE OF INFLORESCENCE BY INTRODUCING GENE FOR PETUNIA TRANSCRIPTION FACTOR PetSPL2

[X] This application claims priority from each of the following Application Nos./filing dates:
JP 10-224852 filed August 7, 1998, the disclosure(s) of which is (are) incorporated by reference.

[X] Please amend this application by adding the following before the first sentence:--This application claims the benefit of Application JP 10-224852 filed August 7, 1998--the disclosure of which is incorporated by reference.--

Enclosed are:
[X] 5 sheet(s) of [x] formal [ ] informal drawing(s); 24 pages of specification including description, claims and abstract; [X] title page.
[ ] An assignment of the invention to _____ .
[X] A [ ] signed [X] unsigned Declaration & Power of Attorney.
[ ] A [ ] signed [ ] unsigned Declaration.
[ ] A Power of Attorney by Assignee with Certificate under 37 CFR 3.73(b).
[ ] A verified statement to establish small entity status under 37 CFR 1.9 and 37 CFR 1.27 [ ] is enclosed [ ] was filed in the prior application and small entity status is still proper and desired.
[X] A certified copy of JP-10-224852
[ ] Information Disclosure Statement under 37 CFR 1.97.
[ ] A petition to extend time to respond in the parent application.
[ ] Notification of change of [ ] power of attorney [ ] correspondence address filed in prior application.
[ ] Please cancel claim(s) _____.
[X] Other: Appendix A, (5 pages) and computer readable copy with transmittal letter.

Pursuant to 37 CFR §1.53(f), Applicant requests deferral of the filing fee until submission of the Missing Parts of Application.

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Respectfully submitted,
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METHOD FOR SHORTENING INTERNODE OF INFLORESCENCE BY INTRODUCING GENE FOR PETUNIA TRANSCRIPTION FACTOR PetSPL2

BACKGROUND OF THE INVENTION

1. FIELD OF THE INVENTION:

The present invention relates to a gene encoding a transcription factor capable of altering characters of a plant and its use. More particularly, the present invention relates to the PetSPL2 gene which is a novel gene derived from *Petunia hybrida*, genes related thereto, and the use thereof.

2. DESCRIPTION OF THE RELATED ART:

In order to clarify regulatory mechanisms controlling the characters of a plant, for example, morphogenesis of a flower, molecular biological and molecular geneticical studies have been conducted using *Arabidopsis thaliana*, *Antirrhinum majus*, and *Petunia hybrida*. In particular, *Petunia hybrida* is preferably used as a subject of studies for the following reasons: high value as a horticultural plant; the presence of various species; ease of transformation; ease to observe due to its large flower; and accumulation of genetical findings (H. Takatsuji, "Molecular mechanism for determining a shape of a plant", Cell Technology, Plant Cell Technology Series (SHUJUNSHA), pp. 96-106).

Genes which cause mutation have been isolated from mutants in which floral organs of the above-mentioned plant is altered. As a result, it is becoming clear that transcription factors play important roles in differentiation and morphogenesis of a flower. For example, SUPERMAN of *Arabidopsis thaliana* is a transcription factor having a zinc finger motif as a DNA binding domain. It is known that, in SUPERMAN mutant with its

gene mutated, number of stamens are remarkably increased, and pistils are defective (THE IDEN, April, 1997 (Vol. 51, No. 4), pp. 34-38).

For understanding the mechanism for the control of characters of a plant, it is important to identify a novel transcription factor which is involved in such control. A gene for a transcription factor which controls morphogenesis of a flower may be introduced into a plant by using gene engineering procedure. It is possible to obtain a plant, using gene introduction, having a flower with novel characters which has not been obtained or is not likely to be obtained by a conventional breeding. It is considered that a plant with such novel characters is horticulturally valuable.

SUMMARY OF THE INVENTION

A gene of the present invention has DNA which is selected from a) or b): a) DNA having a nucleotide sequence from the 190th position to the 807th position of a nucleotide sequence represented in SEQ.ID NO. 1 of Sequence Listing; or b) DNA which hybridizes to DNA of a) under stringent conditions, and encodes a transcription factor capable of altering characters of a plant.

A gene of the present invention encodes a transcription factor which is selected from i) or ii): i) a transcription factor having an amino acid sequence from the 1st position to the 206th position of an amino acid sequence represented in SEQ.ID NO. 2; or ii) a transcription factor having an amino acid sequence in which one or more amino acids of i) are subjected to deletion, substi-

tution, or addition, and being capable of altering characters of a plant.

In one embodiment of the present invention, the characters of a plant include one selected from the group consisting of the height of a plant and the length of an internode.

A method for producing a transgenic plant of the present invention includes the steps of: introducing a plant cell with the above-mentioned gene; and regenerating a plant body from the plant cell having the introduced gene.

In one embodiment of the present invention, the plant belongs to dicotyledon.

In another embodiment of the present invention, the plant belongs to Solanaceae.

In another embodiment of the present invention, the plant belongs to Petunia.

In another embodiment of the present invention, the gene is incorporated into a plant expression vector.

A transgenic plant of the present invention is produced by the above-mentioned method.

Thus, the invention described herein makes possible the advantages of (1) providing a gene encoding a transcription factor capable of altering characters of a plant, in particular, a height of a plant and a length

of an internode; (2) providing a method for producing a plant with its character altered by introduction of the gene; and (3) providing a transgenic plant.

These and other advantages of the present invention will become apparent to those skilled in the art upon reading and understanding the following detailed description with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a nucleotide sequence of PetSPL2 gene and its deduced amino acid sequence.

Figure 2 is a schematic view showing a PetSPL2 high expression vector (pBIN-35S-PetSPL2).

Figure 3 shows an autoradiogram of a denatured agarose gel electrophoresis image detecting mRNA of the PetSPL2 gene in *Petunia hybrida* transformed with the PetSPL2 gene.

Figure 4 shows pictures of morphology of a plant body of wild-type *Petunia hybrida* (right) and that of Petunia hybrida transformed with the PetSPL2 gene (left).

Figure 5 shows pictures of internodes of wild-type *Petunia hybrida* (left) and those of *Petunia hybrida* transformed with the PetSPL2 gene(right).

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Hereinafter, the present invention will be

described in detail.

The term "transcription factor" as used herein refers to a protein which binds to a DNA regulatory region of genes to control the synthesis of mRNA. Some transcription factors are known to have a highly conservative amino acid sequence called zinc finger motif in their DNA binding domains.

A gene of the present invention encodes a transcription factor capable of altering characters of a plant. This gene may have either of the following DNAs:

a) DNA having a nucleotide sequence from the 190th position to the 807th position of a nucleotide sequence represented in SEQ.ID NO. 1 of Sequence Listing; or

b) DNA which hybridizes to DNA of a) under stringent conditions, and encodes a transcription factor capable of altering characters of a plant.

The gene of the present invention may also have DNA which encodes a transcription factor capable of altering characters of a plant, and has a homology of about 60% or more, preferably about 70% or more, more preferably about 80% or more, and still more preferably about 90% or more, with DNA of a).

Preferably, the gene of the present invention may contain DNA of a).

The gene of the present invention may also encode either of the following transcription factors:

i) a transcription factor having an amino acid sequence from the 1st position to the 206th position of

an amino acid sequence represented in SEQ.ID NO. 2; or

ii) a transcription factor having an amino acid sequence in which one or more amino acids of i) are subjected to deletion, substitution, or addition, and being capable of altering characters of a plant.

The number of amino acids subject to deletion, substitution, or addition may be about 130 or less, preferably about 60 or less, more preferably about 30 or less, still more preferably about 20 or less, and stll further more preferably 10 or less.

Preferably, the gene of the present invention may encode the transcription factor of i).

The particularly preferred gene in the present invention is PetSPL2 gene. Figure **1** shows a cDNA sequence (SEQ.ID NO. 1) of this gene and its deduced amino acid sequence (SEQ.ID NO. 2).

Alterations in "characters of a plant" refer to any changes in at least one character of a plant. The character of a plant includes, but is not limited to, at least one of the height of a plant and the length of an internode of a plant. These changes are evaluated by comparing the characters of a plant obtained by introducing a gene of the present invention with the characters of a plant (wild-type or horticultural type) before introducing the gene.

Examples of height change of a plant include, but are not limited to, a dwarf and a semi-dwarf. The dwarfism is preferably about 1/2 or less, more preferably

about 1/3 or less of a standard height of a plant before introducing the gene.

An example of length change of an internode includes, but is not limited to, a reduction of an internode. The reduction of an internode includes any reduction of an internode of a reproductive branch (i.e., inflorescence) and an internode of a vegetative branch (i.e., phyllotaxis). The reduction of inflorescence is a particularly preferable example of a change. The change in length of an internode preferably acheieves a length of about 1/2 or less, more preferably about 1/5 or less, and most preferably about 1/10 or less, compared with a standard internode of the plant before introducing a gene.

An example of changed characters of a plant is a combination of a drawf and a reduction of an internode, more preferably a combination of a drawf and a reduction of an internode of an inflorescence.

The gene of the present invention can be isolated, for example, by performing polymerase chain reaction (PCR) with genomic DNA of a plant as a template, using a pair of degenerated primers corresponding to a conserved region of the amino acid sequence encoded by a gene of a known transcription factor, and screening a genomic library of the same plant, using the amplified DNA fragment thus obtained as a probe. Examples of a pair of primers include a combination of 5'-CARGCNYTNGGNGGNCAY-3' (SEQ.ID NO. 3) or 5'-YTNGGNGGNCAYATGAAY-3' (SEQ.ID NO. 4) with 5'-ARNCKNARYTCNARRTC-3' (SEQ.ID NO. 5) in which N is inosine, R is G or A, Y is C or T, and K is T or G.

PCR can be performed in accordance with the manufacturer's instructions for a commercially available kit and instruments, or by a procedure well known to those skilled in the art. A method for producing a gene library, stringent conditions used for hybridization with a probe, and a method for cloning a gene are well known to those skilled in the art. For example, see Maniatis et al., Molecular Cloning, A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989).

A nucleotide sequence of the gene thus obtained can be determined by a nucleotide sequence analysis method known in the art or by a commercially available automatic sequencer.

The gene of the present invention is not limited to those isolated from native genome but may include synthetic polynucleotides. Synthetic polynucleotides can be obtained, for example, by modifying a sequenced gene as described above using a procedure well known to those skilled in the art.

The gene of the present invention can be ligated to an appropriate plant expression vector by a method well known to those skilled in the art and introduced into a plant cell by a known gene recombination technique. The introduced gene is incorporated into the DNA of a plant cell. The DNA of a plant cell includes DNA contained in various organelles (e.g., mitochondria, chloroplasts, etc.) of a plant cell, as well as chromosomes.

The "plant" includes both monocotyledon and dicotyledon. The preferred plant is dicotyledon. The dicotyledon includes both *Archichlamiidae* and *Sympetalidae*. A plant of *Sympetalidae* is preferable. Examples of the plants of *Sympetalidae* include *Gentianales*, *Solanales*, *Lamiales*, *Callitrichales*, *Plantaginales*, *Campanulales*, *Scrophulariales*, *Rubiales*, *Dipsacales*, *Asterales*, and the like. A plant of *Solanales* is preferable. Examples of the plants of *Solanales* include *Solanaceae*, *Hydrophyllaceae*, *Polemoniaceae*, *Cuscutaceae*, *Convolvulaceae*, and the like. *Solanaceae* is preferable. *Solanaceae* includes *Petunia*, *Datura*, *Nicotiana*, *Solanum*, *Lycopersicon*, *Capsicum*, *Physalis*, and *Lycium*, etc. Plants of *Petunia*, *Datura*, and *Nicotiana* are preferable. *Petunia* is more preferable. Examples of the plants of *Petunia* include *P. hybrida*, *P. axillaris*, *P. inflata*, *P. violacea*, and the like. A plant of *P. hybrida* is especially preferable. The "plant" refers to a plant body having a flower and/or a fruit and a seed obtained from it, unless otherwise specified.

Examples of the "plant cell" include cells from plant organs such as leaves and roots, callus, and suspension cultured cells.

The term "plant expression vector" as used herein refers to a nucleic acid sequence in which various regulatory elements, such as a promotor, for regulating expression of the gene of the present invention, are linked to each other so as to be operable in a host plant cell. Preferably, the plant expression vector may include a plant promoter, a terminator, a drug resistant

gene and an enhancer. It is well known to those skilled in the art that a type of the plant expression vector and regulator elements may be varied depending on the type of host cell. A plant expression vector used according to the present invention may further contain a T-DNA region. The T-DNA region allows a gene to be efficiently introduced to plant genome especialy when *Agrobacterium* is used to transform a plant.

The term "plant promoter" as used herein refers to a promoter that functions in a plant. Constitutive promoters as well as tissue-specific promoters which selectively function in a part of a plant body, including a flower, are preferable. Examples of plant promoters include, but are not limited to, Cauliflower mosaic virus (CaMV) 35S promoter and a promoter of nopaline synthase.

The term "terminator" as used herein refers to a sequence positioned downstream of a region of a gene encoding a protein, which is involved in the termination of transcription of mRNA, and the addition of a poly A sequence. The terminator is known to contribute to the stability of mRNA, thereby affecting the expression level of a gene. Examples of such terminators include, but are not limited to, CaMV 35S terminator and a terminator of a nopaline synthase gene (Tnos).

A "drug resistant gene" is desirable to facilitate the selection of transgenic plants. The examples of such drug resistant genes for use in the invention include, but are not limited to, a neomycin phosphotransferase II (NPTII) gene for conferring kanamycin resistance, and a hygromycin phosphotransferase gene

for conferring hygromycin resistance.

An "enhancer" may be used to enhance the expression level of a gene of interest. As the enhancer, an enhancer region containing a sequence upstream of the above-mentioned CaMV 35S promoter is preferable. More than one enhancers may be used in one plant expression vector.

The plant expression vector according to the present invention may be produced by using a recombinant DNA technique well known to those skilled in the art. The examples of preferable vectors for constructing a plant expression vector include, but are not limited to pBI-type vectors or pUC-type vectors.

A plant expression vector may be introduced into a plant cell by using methods well known to those skilled in the art, for example, a method of infecting a plant cell with *Agrobacterium* or a method of directly introducing a vector into a cell. The method using *Agrobacterium* may be performed, for example, as described in Nagel et al., *Microbiol. Lett.*, 67, 325, 1990. According to this method, *Agrobacterium* is first transformed with a plant expression vector by, for example, electroporation, and then the transformed *Agrobacterium* is infected to a plant cell by a well-known method such as a leaf-disk method. Examples of the methods for directly introducing a plant expression vector into a cell include, but are not limited to, an electroporation method, a particle gun method, a calcium phosphate method, and a polyethylene glycol method. These methods are well known in the art and a method suitable for a

particular plant to be transformed may be suitably selected by those skilled in the art.

The cells in which plant expression vectors have been introduced are selected based on their drug resistance such as resistance to kanamycin. Thereafter, the cells may be regenerated to a plant body by using a conventional method.

Expression of the introduced gene of the present invention in the regenerated plant body can be confirmed by using a procedure well known to those skilled in the art. This confirmation can be performed by northern blot analysis, for example. More specifically, the total RNAs may be extracted from leaves of a resultant plant, and may be subjected to denatured agarose gel electrophoresis, and then, RNAs may be blotted onto an appropriate membrane. The blot can be hybridized with a labelled RNA probe complementary to a part of the introduced gene to detect mRNA from the gene of the present invention.

The plant of the present invention is a transgenic plant produced by the above-mentioned procedure. It is preferable that the altered characters of the transgenic plant (i.e., a height of a plant and/or a length of an internode) include that which is not found in a known wild-type or horticultural type. It is also preferable that the altered characters of a plant are horticulturally valuable. Furthermore, it is preferable that altered characters of a plant are stably conserved over subsequent generations.

EXAMPLES

Hereinafter, the present invention will be described by way of the following illustrative examples. Restriction enzymes, plasmids and the like used in the following examples are available from commercial sources.

(Example 1: Isolation of PetSPL2 gene)

The protein encoded by the SUPERMAN gene of *Arabidopsis thaliana* was compared with the protein encoded by the GmN479 gene (Kouchi et al., personal communication) expressed specifically in soy bean root nodules. Three different degenerate primers for use in PCR were synthesized based on the amino acid sequences commonly present in both proteins. The nucleotide sequences of two primers oriented 5' to 3' in the genes are 5'-CARGCNYTNGGNGGNCAY-3' (primer 1, corresponding to an amino acid sequence QALGGH; SEQ ID NO: 3) and 5'-YTNGGNGGNCAYATGAAY-3' (Primer 2, corresponding to an amino acid sequence LGGHMN; SEQ ID NO: 4), respectively, and a nucleotide sequence of a primer oriented 3' to 5' in the genes is 5'-ARNCKNARYTCNARRTC-3' (primer 3, corresponding to an amino acid sequence DLELRL; SEQ ID NO:5), wherein N is inosine, Y is either C or T, R is either G or A, and K is either T or G.

A first set of PCR was conducted with primer 1 and primer 3 under the following conditions: 94°C for 10 minute, followed by 30 cycles of 94°C for 30 seconds, 50°C for 30 seconds and 72°C for 60 seconds, and subsequently 72°C for 7 minutes, using as a template a genomic DNA of a petunia (*Petunia hybrida var. Mitchell*) extracted according to the method described in Boutry, M. and Chua N. H. (1985) EMBO J. 4, 2159-2165. In addition, a second PCR was conducted with primer 2 and primer 3,

while using as a template a portion of the product from the first PCR. The reaction conditions were the same as those used in the first PCR. Amplified DNA fragments were inserted into the TA cloning vector (produced by Invitrogen), which were then introduced into *E. coli* according to a conventional method. Plasmids were extracted from the transformed *E. coli* and the nucleotide sequences of the DNA fragment was determined. The results revealed that a part of zinc finger motif contained in common in SUPERMAN and GmN479 was encoded within the resulting DNA fragment. The gene from which this DNA fragment was derived was designated as PetSPL2 gene. In the same series of experiments, the presence of 3 other DNAs (PetSPL1, 3 and 4 genes) containing a nucleotide sequence similar to that of PetSPL2 was demonstrated. For details regarding the PetSPL3 gene, see Japanese Patent Application No. 10-65921.

To clone cDNA of the PetSPL2 gene, the DNA fragment described above and a GENETRAP cDNA selection kit (produced by BRL) were used to screen a cDNA library of petunia floral buds (*Petunia hybrida var. Mitchell*) which had been created, using the BRL kit, within a pSPORT plasmid vector (produced by BRL). Several clones for the PetSPL2 gene were obtained by screening this cDNA library. Thus, cDNA for the PetSPL2 gene derived from Petunia was isolated.

(Example 2: Analysis of the nucleotide sequence and amino acid sequence of PetSPL2 gene)

The longest clone out of the clones obtained in Example 1 contained a PetSPL2 gene cDNA fragment of about 1.0kb. The DNA nucleotide sequence of this cDNA fragmet

was determined (SEQ ID NO: 1). From an open reading frame contained in the resulting DNA nucleotide sequence, an amino acid sequence of the protein was deduced (SEQ ID NO: 2).

The comparison of the nucleotide sequences indicated that the PetSPL2 gene showed 58%, 67% and 51% nucleotide sequence homology to the SUPERMAN, PetSPL1 and PetSPL4 genes, respectively. The PetSPL2 gene showed 52% nucleotide sequence homology to PetSPL3 gene. This comparison of the nucleotide sequences was conducted only within the coding region of each gene.

The deduced amino acid sequence of PetSPL2 contained a single TFIIIA-type zinc finger motif similar to that of SUPERMAN. On this basis, it was presumed that PetSPL2 was a transcription factor. PetSPL2 showed about 37% and 23% homology to SUPERMAN and PetSPL3, respectively, in the full-length amino acid sequence.

Table 1 compares the amino acid sequence of SUPERMAN with that of each PetSPL in the zinc finger motif. Amino acid homology (about 100%) of PetSPL2 to SUPERMAN in the zinc finger motif was shown to be the same as the corresponding homology (about 100%) of PetSPL1 to SUPERMAN and to be higher compared with that (about 76%) of PetSPL3 to SUPERMAN (wherein the amino acid sequence homology was calculated assuming that the zinc finger motif extends from the 4th C to the 24th H). Table 1 also shows comparison of C terminal hydrophobic region of SUPERMAN with that of each PetSPL.

Table 1

Zinc-finger

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SUPERMAN      SYTCSFCKREFRSAQALGGHMNVHRRDRARLRLQQSPSSSSTP
PetSPL1       SYTCSFCKREFRSAQALGGHMNVHRRDRARLRL-QSPPRENGT
PetSPL2       SYTCSFCKREFRSAQALGGHMNVHRRDRAILR--QSPPRDINR
PetSPL3       SYECNFCKRGFSNAQALGGHMNIHRKDKAKLKKQKQHQRQQKP
PetSPL4       FYRCSFCKRGFSNAQALGGHMNIHRKDRAKLREISTDNLNIDQ
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C-terminal hydrophobic region

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                    *       * *       *       *
SUPERMAN      ILRNDEIISLELEIGLINESEQDLDLELRLGFA
PetSPL1       LMKRSEFLRLELGIGMINESKEDLDLELRLGYT
PetSPL2       VIKKSEFLRLDLGIGLISESKEDLDLELRLGST
PetSPL3       GSVDSRENRLPARNQETTPFYAELDLELRLGHE
PetSPL4       CGTLDEKPKRQAENNDMQQDDSKLDLELRLGPD
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From the results described above, it was presumed that PetSPL2 is a novel transcription factor with a closer relationship to SUPERMAN than to PetSPL3, and belongs to a class different from that of PetSPL3.

(Example 3: Construction of a plant expression vector containing a polynucleotide encoding PetSPL2)

A DNA fragment (HindIII-XbaI fragment) containing a CaMV 35S promoter in a plasmid pBI221 (purchased from Clontech) and a DNA fragment (SacI-EcoRI fragment) containing a NOS terminator were sequentially inserted into a multicloning site of plasmid pUCAP (van Engelen, F. A. et al., Transgenic Res. 4:288-290 (1995)) to produce pUCAP35S. On the other hand, pSPORT/PetSPL2

plasmid containing PetSPL2 was cleaved at KpnI and SacI sites (which are the sites within this vector), and inserted between KpnI and SacI sites of the pUCAP35. This recombinant plasmid was further cleaved with AscI and PacI, and the resultant DNA fragment encoding PetSPL2 was introduced into AscI and PacI sites of a binary vector pBINPLUS (van Engelen, F. A. et al., (1995), supra).

The constructed PetSPL2 gene high expression vector (pBIN-35S-PetSPL2) includes, as shown in Figure 2a, a CaMV 35S promoter region (P35S; 0.9kb), a polynucleotide of the present invention encoding PetSPL2 (PetSPL2; 1.0kb) and a terminator region of nopaline synthase (Tnos; 0.3kb). In Figure 2, Pnos and NPTII indicate a promoter region of nopaline synthase and neomycin phosphotransferase II gene, respectively. LB and RB indicate T-DNA left border and T-DNA right border, respectively.

(Example 4: Introduction of the PetSPL2 gene into petunia cells)

(1)(Transformation of *Agrobacterium tumefaciens*)

Agrobacterium tumefaciens LBA4404 line (purchased from Clontech) was cultured in an L medium containing 250 µg/ml streptomycin and 50 µg/ml rifampicin at 28°C. According to the method of Nagel et al. (1990) (supra), a cell suspension of this strain was prepared. The PetSPL2 gene high expression vector constructed in Example 3 was introduced into the above described strain by electroporation.

(2)(Introduction of a polynucleotide encoding PetSPL2 into Petunia cell)

The Agrobacterium tumefaciens LBA4404 line obtained in (1) was cultured (at 28°C, 200 rpm) with agitation in YEB medium (D. M. Glover ed. DNA Cloning, IPL PRESS, second edition, p.78), followed by a 20-fold dilution with sterilized water. Leaf sections of petunia (*Petunia hybrida var. Mitchell*) were cultured in this diluted solution. After 2-3 days, the *Agrobacterium* was removed using a medium containing carbenicillin, and thereafter these leaf sections were subcultured in a selection medium by transferring to new media every 2 weeks. The Kanamycin resistance trait conferred by the expression of the NPTII gene derived from pBINPLUS, introduced together with the above-mentioned PetSPL2 gene, was used as an indicator to select transformed petunia cells. Callus was induced from the transformed cells using a conventional method, and then re-differentiated into a plant body.

(Example 5: Expression of the PetSPL2 gene in a PetSPL2 transformed plant)

Total RNAs were extracted from leaves of 14 PetSPL2 transformed petunias obtained in Example 4. 10 µg each of the extracts was subjected to denatured agarose gel electrophoresis, and blotted onto a Genescreen plus filter (produced by DuPont) in accordance with a conventional method. A PetSPL2 antisense RNA was labelled using DIG RNA labelling kit (produced by Boeringer Mannheim). Hybridization and filter washing were performed with the labelled RNA according to the instructions of the kit. After the washing, the filter was exposed to an XAR film (produced by Kodak) for 1 hour at room temperature. Figure 3 shows an autoradiogram of an image of denatured agarose gel electrophoresis which detected PetSPL2 gene

mRNAs from 13 petunias. These results indicated that 4 out of 13 individual transformant petunias expressed PetSPL2 mRNA at a high level under the control of a high expression promoter.

Example 6: Phenotype of a transformant petunia expressing PetSPL2 gene at high level

Phenotypes as described below were commonly observed in 3 petunias out of 4 individual transformant petunias expressing the PetSPL2 gene at high levels. The remaining one petunia expressed the PetPSL2 gene at relatively low level compared with the above three petunias. The most significant change observed in the plant bodies was shortening of the internode length of their inflorescences (i.e., suppression of internode elongation) and dwarfism associated therewith (Figure 4; left panel shows a PetSPL2-transformant petunia and right panel shows a wild type petunia). This change was observed more extensively in the reproductive stage, namely inflorescence, than in the vegetative stage. The internode length of the inflorescence was shown to be less than one tenth of the wild type (Figure 5; left panel shows internodes of a wild type petunia and right panel shows those of a PetSPL3-transformant petunia). Other changes were rounding of leaves, a moderate decrease in size of flowers and the like (Figure 4).

For a gene involved in controlling the internode elongation of inflorescence, an ERECTA gene of *Arabidopsis thaliana* has been reported (Torii et al., 1996, Plant Cell, 8:735). However, no significant homology between the ERECTA gene and the PetSPL2 gene of the present invention is found at either a nucleotide se-

quence level or an amino acid sequence level. Some of the genes which are involved in plant hormone synthesis and control thereof (rolA, etc.) are also known to shorten the internode length. However, these plant hormone-related genes are known for exhibiting multiple effects as well as controlling the internode elongation (Dehio et al., 1993, Plant Mol. Bio., 23:1199).

From the results described above, it has been shown that the PetSPL2-transformant petunia becomes more dwarfish with shortening of the internode length such that the appearance of the flowers change significantly as compared to the wild types. Accordingly, it is understood that introduction of the PetSPL2 gene is useful, especially for ornamental flowers or horticultral types whose internode length is prone to elongation. A significant change in the appearance of flowers may confer a new value for appreciation on plants. In addition, suppression of the height of a plant may have a significant horticultural value with respect to making the plant resistant to dislodging. Furthermore, fruit trees having the PetSPL gene introduced therein are expected to become compact in their shapes. This is meaningful because it may make fruit-harvesting work more efficient.

According to the present invention, a gene encoding a transcription factor capable of altering morphology, and the like of a plant is provided. By utilizing the present gene, a plant with altered character can be produced. The generated plant is horticulturally useful because it is provided with the character which is not found or rarely found in a wild-type and a horticultural type.

Various other modifications will be apparent to and can be readily made by those skilled in the art without departing from the scope and spirit of this invention. Accordingly, it is not intended that the scope of the claims appended hereto be limited to the description as set forth herein, but rather that the claims be broadly construed.

WHAT IS CLAIMED IS:

1. A gene having DNA which is selected from a) or b):

a) DNA having a nucleotide sequence from the 190th position to the 807th position of a nucleotide sequence represented in SEQ.ID NO. 1 of Sequence Listing; or

b) DNA which hybridizes to DNA of a) under stringent conditions, and encodes a transcription factor capable of altering characters of a plant.

2. A gene encoding a transcription factor which is selected from i) or ii):

i) a transcription factor having an amino acid sequence from the 1st position to the 206th position of an amino acid sequence represented in SEQ.ID NO. 2; or

ii) a transcription factor having an amino acid sequence in which one or more amino acids of i) are subjected to deletion, substitution, or addition, and being capable of altering characters of a plant.

3. A gene according to claim 1, wherein the characters of a plant include one selected from the group consisting of a height of a plant and a length of an internode.

4. A method for producing a transgenic plant, comprising the steps of:

introducing a plant cell with the gene of claim 1; and

regenerating a plant body from the plant cell having the introduced gene.

5. A method according to claim 4, wherein the plant

belongs to dicotyledon.

6. A method according to claim 5, wherein the plant belongs to Solanaceae.

7. A method according to claim 6, wherein the plant belongs to Petunia.

8. A method according to claim 4, wherein the gene is incorporated into a plant expression vector.

9. A transgenic plant produced by the method of claim 4.

ABSTRACT OF THE DISCLOSURE

A gene encoding DNA which is selected from a) or b): a) DNA having a nucleotide sequence from the 190th position to the 807th position of a nucleotide sequence represented in SEQ.ID NO. 1 of Sequence Listing; or b) DNA which hybridizes to DNA of a) under stringent conditions, and encodes a transcription factor capable of altering characters of a plant.

PATENT

Attorney Docket No. 085761-0004

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	)	
	)	
Hiroshi TAKATSUJI et al.	)	Examiner: Unassigned
	)	
Application No.: Unknown	)	Art Unit:
	)	
Filed: Herewith	)	TRANSMITTAL OF SEQUENCE LISTING
	)	
For: METHOD FOR SHORTENING INTERNODE OF INFLORESCENCE BY INTRODUCING GENE FOR PETUNIA TRANSCRIPTION FACTOR PetSPL2	)	

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Applicants hereby submit a Sequence Listing and a Sequence Listing in Computer readable form. Applicants' attorneys attest that the information in the "Sequence Listing" is identical to the written sequence listing as requested under 37 CFR 1.821 (f).

Respectfully submitted,

William M. Smith
Registration No. 30,223

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(415) 326-2400
Fax (415) 576-0300

SEQUENCE LISTING

<110> NATIONAL INSTITUTE OF AGROBIOLOGICAL RESOURCES, MINISTRY OF AGRICULTURE, FORESTRY AND FISHERIES
<120> METHOD FOR SHORTENING INTERNODE OF INFLORESCENCE BY INTRODUCING GENE FOR PETUNIA TRNSCRIPTION FACTOR PetPSL2
<130> J198412287
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TGAACATAAT TATTATACTT ACTATTAAGC TAGATTTCCT CTTGATCTTG CTAGGTTTGA 180
CTGGAGAAA ATG GCA GGC ATG GAT AGA AAC AGT TTC AAC AGT AAG TAC TTC 231
          Met Ala Gly Met Asp Arg Asn Ser Phe Asn Ser Lys Tyr Phe
                                  5                  10
AAA AAC AAA AGC ATC ATG GCA AGA CAG ATG GAG TAC TTG AAT AAC AAC   279
Lys Asn Lys Ser Ile Met Ala Arg Gln Met Glu Tyr Leu Asn Asn Asn
15                  20                  25                  30
AAT GGC GAC AAT AAC AAC AAC AAT AAT GTT ACA AGC TCA TTA CGA GAT   327
Asn Gly Asp Asn Asn Asn Asn Asn Asn Val Thr Ser Ser Leu Arg Asp
                35                  40                  45
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Asn Tyr Gly Asn Glu Asp His Leu Leu Gly Gly Leu Phe Ser Trp Pro
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Pro Arg Ser Tyr Thr Cys Ser Phe Cys Lys Arg Glu Phe Arg Ser Ala
65 70 75
CAA GCT CTT GGT GGA CAC ATG AAT GTT CAT AGA AGA GAT AGA GCC ATT 471
Gln Ala Leu Gly Gly His Met Asn Val His Arg Arg Asp Arg Ala Ile
80 85 90
TTG AGA CAA TCA CCA CCT AGA GAT ATT AAT AGG TAT TCT CTT CTA AAC 519
Leu Arg Gln Ser Pro Pro Arg Asp Ile Asn Arg Tyr Ser Leu Leu Asn
95 100 105 110
CTT AAT CTT GAA CCA AAC CCT AAC TTT TAC CCT AGT CAT AAC CCT AGT 567
Leu Asn Leu Glu Pro Asn Pro Asn Phe Tyr Pro Ser His Asn Pro Ser
115 120 125
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Phe Ser Arg Lys Phe Pro Pro Phe Glu Met Arg Lys Leu Gly Lys Gly
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GTT GTT CCA AAC AAT CAC TTG AAA AGT GCC AGA GGG CGT TTT GGA GTT 663
Val Val Pro Asn Asn His Leu Lys Ser Ala Arg Gly Arg Phe Gly Val
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GAG AAA ATT GAC TCT TTC ATG CAA GAA AAA GAA TGT ACT ACT ACA GTG 711
Glu Lys Ile Asp Ser Phe Met Gln Glu Lys Glu Cys Thr Thr Thr Val
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Ile Lys Lys Ser Glu Phe Leu Arg Leu Asp Leu Gly Ile Gly Leu Ile
175 180 185 190
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Asp Asn Asn Asn Asn Asn Asn Val Thr Ser Ser Leu Arg Asp Asn Tyr
        35                  40                  45
Gly Asn Glu Asp His Leu Leu Gly Gly Leu Phe Ser Trp Pro Pro Arg
    50                  55                  60
Ser Tyr Thr Cys Ser Phe Cys Lys Arg Glu Phe Arg Ser Ala Gln Ala
65                  70                  75                  80
Leu Gly Gly His Met Asn Val His Arg Arg Asp Arg Ala Ile Leu Arg
                85                  90                  95
Gln Ser Pro Pro Arg Asp Ile Asn Arg Tyr Ser Leu Leu Asn Leu Asn
            100                 105                 110
Leu Glu Pro Asn Pro Asn Phe Tyr Pro Ser His Asn Pro Ser Phe Ser
        115                 120                 125
Arg Lys Phe Pro Pro Phe Glu Met Arg Lys Leu Gly Lys Gly Val Val
    130                 135                 140
Pro Asn Asn His Leu Lys Ser Ala Arg Gly Arg Phe Gly Val Glu Lys
145                 150                 155                 160
Ile Asp Ser Phe Met Gln Glu Lys Glu Cys Thr Thr Thr Val Ile Lys
                165                 170                 175
Lys Ser Glu Phe Leu Arg Leu Asp Leu Gly Ile Gly Leu Ile Ser Glu
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FIG. 1

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TGAACATAAT TATTATACTT ACTATTAAGC TAGATTTCCT CTTGATCTTG CTAGGTTTGA

CTGGAGAAAA TGGCAGGCAT GGATAGAAAC AGTTTCAACA GTAAGTACTT CAAAAACAAA
          M   A G M   D R N   S F N S   K Y F   K N K

AGCATCATGG CAAGACAGAT GGAGTACTTG AATAACAACA ATGGCGACAA TAACAACAAC
S I M A   R Q M   E Y L   N N N N   G D N   N N N

AATAATGTTA CAAGCTCATT ACGAGATAAT TATGGAAATG AAGATCATTT ACTTGGTGGA
N N V T   S S L   R D N   Y G N E   D H L   L G G

CTATTCTCTT GGCCTCCAAG ATCTTATACA TGTAGCTTTT GTAAAAGGGA ATTTAGATCT
L F S W   P P R   S Y T   C S F C   K R E   F R S

GCTCAAGCTC TTGGTGGACA CATGAATGTT CATAGAAGAG ATAGAGCCAT TTTGAGACAA
A Q A L   G G H   M N V   H R R D   R A I   L R Q

TCACCACCTA GAGATATTAA TAGGTATTCT CTTCTAAACC TTAATCTTGA ACCAAACCCT
S P P R   D I N   R Y S   L L N L   N L E   P N P

AACTTTTACC CTAGTCATAA CCCTAGTTTT TCAAGAAAAT TCCCACCTTT TGAAATGAGG
N F Y P   S H N   P S F   S R K F   P P F   E M R

AAATTAGGAA AAGGAGTTGT TCCAAACAAT CACTTGAAAA GTGCCAGAGG GCGTTTTGGA
K L G K   G V V   P N N   H L K S   A R G   R F G

GTTGAGAAAA TTGACTCTTT CATGCAAGAA AAAGAATGTA CTACTACAGT GATCAAGAAG
V E K I   D S F   M Q E   K E C T   T T V   I K K

TCCGAGTTTC TAAGATTGGA CTTGGGAATT GGGTTGATCA GTGAATCAAA GGAAGATTTA
S E F L   R L D   L G I   G L I S   E S K   E D L

GATCTTGAAC TTCGACTGGG ATCCACTTAA CTATATCTAA TTTTTACGGC ATTAAGGTTT
D L E L   R L G   S T

GTAAATTGAG TCGACAGCTT AGTCAAAACT ACTTATGCAC TTTAATATGG CTTCTTGTGC

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FIG. 2

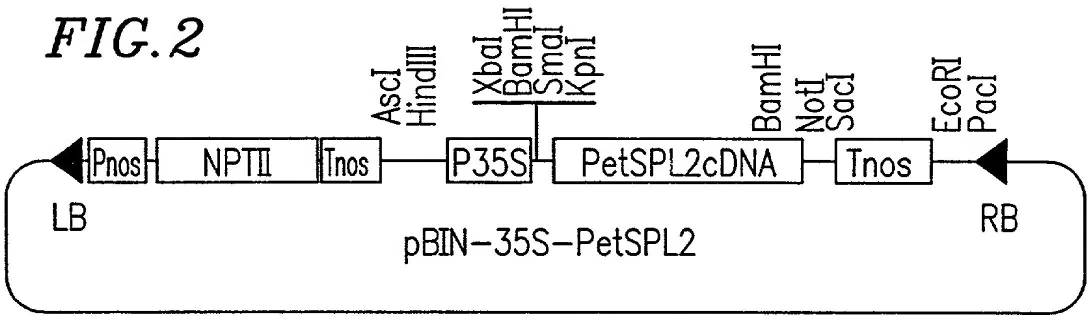
AscI
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FIG.3

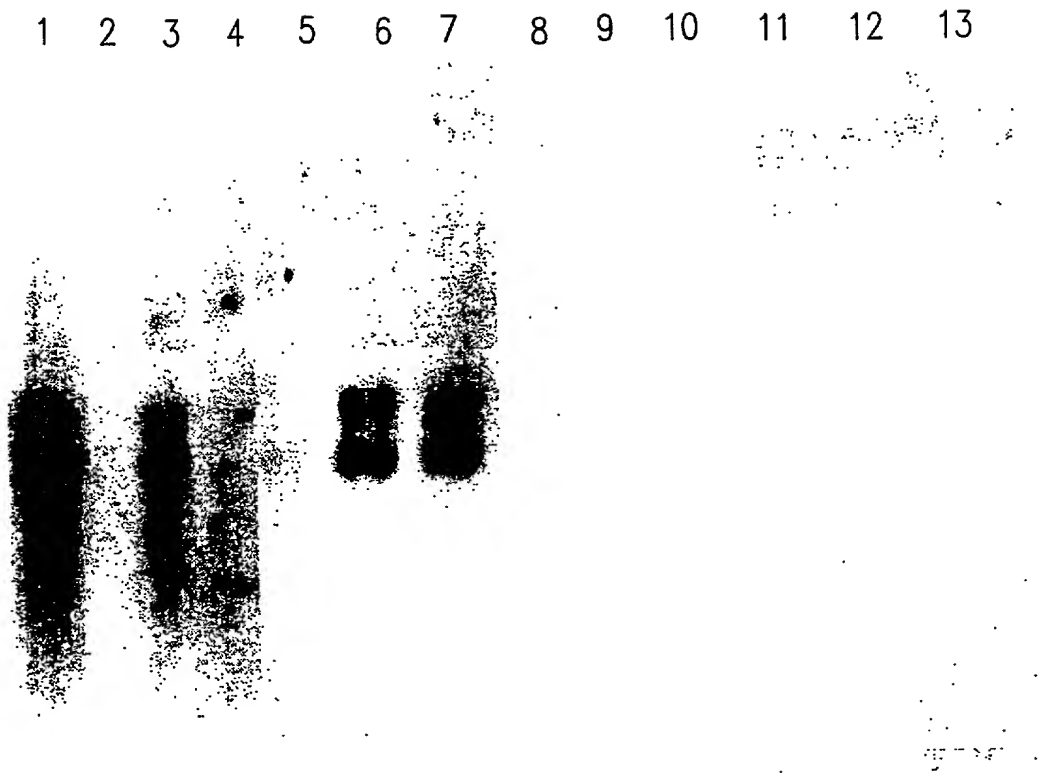

FIG. 4

FIG. 5

Attorney Docket No. 085761-0004

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I declare that:

My residence, post office address and citizenship are as stated below next to my name; I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: **METHOD FOR SHORTENING INTERNODE OF INFLORESCENCE BY INTRODUCING GENE FOR PETUNIA TRANSCRIPTION FACTOR PetSPL2,** the specification of which X is attached hereto.

I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56. I claim foreign priority benefits under Title 35, United States Code, Section 119 of any foreign applications(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)

Country	Application No.	Date of Filing	Priority Claimed Under 35 USC 119
JAPAN	10-224852	AUGUST 7, 1998	Yes X No _
			Yes _ No _

I hereby claim the benefit under Title 35, United States Code §119(e) of any United States provisional application(s) listed below:

Application No.	Filing Date

I claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Section 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application No.	Date of Filing	Status
		_ Patented _ Pending _ Abandoned
		_ Patented _ Pending _ Abandoned

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

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MARGARET A. POWERS, Reg. No. **39,804**
JOE LIEBESCHUETZ, Reg. No. **37,505**

Send Correspondence to:	Direct Telephone Calls to: (Name, Reg. No., Telephone No.)
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Full Name of Inventor 2	Last Name **NAKAGAWA**	First Name **HITOSHI**	Middle Name or Initial	
Residence & Citizenship	City **IBARAGI**	State/Foreign Country **JAPAN**	Country of Citizenship **JAPAN**	
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Full Name of Inventor 3	Last Name	First Name	Middle Name or Initial	
Residence & Citizenship	City	State/Foreign Country	Country of Citizenship	
Post Office Address	Post Office Address	City	State/Country	Zip Code

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signature of Inventor 1	Signature of Inventor 2	Signature of Inventor 3
Hiroshi Takatsuji	Hitoshi Nakagawa	
Date	Date	Date

DP.MRG 8/96